A novel *dnaK* operon from *Porphyromonas gingivalis*

Akihiro Yoshida[a,b], Yoshio Nakano[b,*], Yoshihisa Yamashita[b], Takahiko Oho[b], Yukie Shibata[b], Masamichi Ohishi[a], Toshihiko Koga[b]

[a]*First Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[b]*Department of Preventive Dentistry, Faculty of Dentistry, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

Received 18 December 1998; received in revised form 8 February 1999

Abstract The nucleotide sequence of the *dnaK* operon cloned from *Porphyromonas gingivalis* revealed that the operon does not contain homologues of either *dnaJ* or *grpE*. However, there were two genes which encode small heat shock proteins immediately downstream from the *dnaK* and they were transcribed together with *dnaK* as one unit. The ATPase activity of the *P. gingivalis* DnaK was synergistically stimulated up to 40-fold in the simultaneous presence of *Escherichia coli* DnaJ and GrpE. These results suggest that the DnaK homologue of *P. gingivalis*, with its unique genetic structure and evolutionary features, works as a member of the DnaK chaperone system.

Key words: *dnaK* operon; Heat shock protein; Molecular chaperone; Small heat shock protein; *Porphyromonas gingivalis*

1. Introduction

Heat shock proteins (HSPs) or stress proteins are produced by prokaryotic and eukaryotic cells in response to a variety of physiological insults [1]. HSPs belonging to the HSP90, HSP70 and HSP60 families are among the most highly conserved and abundant proteins found in living prokaryotic and eukaryotic organisms [1,2]. It has recently become clear that members of the HSP families, mainly HSP60 and HSP70, which were originally characterized in *Escherichia coli* as GroEL and DnaK, respectively, are major members of molecular chaperones and involved in folding and degradation of proteins [3–5]. The best characterized folding modulators in the cytoplasm of *E. coli* are the DnaK-DnaJ-GrpE and GroEL-GroES molecular chaperone systems [6]. Neither DnaK nor GroEL acts as a chaperone alone, they require co-chaperones [7]. In the DnaK chaperone system, DnaJ (41 kDa) and GrpE (22 kDa) are required for the chaperone activity of DnaK in vivo and in vitro [8].

Recently, we found a novel type of *dnaK* operon, lacking both *dnaJ* and *grpE* genes but containing two small heat shock genes, from *Porphyromonas gingivalis*. *P. gingivalis* is a Gram-negative black-pigmented anaerobe associated with several periodontal diseases including adult periodontitis, generalized juvenile periodontitis, periodontal abscesses and refractory periodontitis [9]. It is possible that the molecular chaperone activity of DnaK in *P. gingivalis* is important in enabling this organism to cope with the potentially detrimental effects of environmental stressors. Analysis of *dnaK* expression and DnaK function in *P. gingivalis* should contribute to an understanding of the mechanism by which *P. gingivalis* withstands environmental extremes during inflammation at periodontal sites. In this study, the *P. gingivalis dnaK* operon was cloned and the promoter regions and the transcriptional unit were identified. In addition, the interaction between the recombinant DnaK of *P. gingivalis* and *E. coli* DnaJ/GrpE if *E. coli* was analyzed.

*Corresponding author. Fax: (81) (92) 642 6354.
E-mail: yosh@dent.kyushu-u.ac.jp

Abbreviations: aa, amino acid(s); IPTG, isopropyl-β-D-thiogalactopyranoside; ORF, open reading frame; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-mediated PCR; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

The nucleotide sequence reported here was submitted to DDBJ/EMBL/GenBank databases and assigned accession number AB015879.

2. Materials and methods

2.1. Bacterial strains

P. gingivalis ATCC 33277, *E. coli* DH5α and *E. coli* ER2566 were used.

2.2. DNA and RNA manipulations

DNA and RNA manipulations were carried out according to standard protocols [10]. Chromosomal DNA was isolated from *P. gingivalis* cells by the guanidine isothiocyanate method with the IsoQuick DNA extraction kit (MicroProbe). Total RNA was prepared from *P. gingivalis* ATCC 33277 using a FastPrep Device (BIO 101) in combination with FastRNA kit-BLUE (BIO 101) according to the supplier's protocol with a minor modification.

2.3. Cloning of the dnaK gene of P. gingivalis

To detect the *dnaK* gene homologues, we constructed a DIG-labelled PCR probe (Boehringer GmbH, Mannheim) in accordance with the manufacturer's instructions. The probe was generated by PCR amplification of *P. gingivalis* genomic DNA using degenerate oligo primers (5′-CCNGCNTAYTTYAAYGAY-3′ and 5′-AGNACRTCYTTNACRTCN-3′). A cosmid gene bank of *P. gingivalis* ATCC 33277 was constructed with chromosomal DNA partially digested with *Bam*HI and the *Bam*HI-digested cosmid vector pMBLcos [11]. Packaging and transfection of pMBLcos and insert fragments were performed using bacteriophage λ (Gigapack II XL, Stratagene) and *E. coli* DH5α. The *P. gingivalis* clone bank was screened by colony hybridization [10].

2.4. Primer extension analysis of transcripts

Primer extension reactions were performed as described by Sambrook et al. [10]. For *dnaK* transcript analysis, a 24 bp synthetic oligonucleotide was chosen, which is able to hybridize to the nucleotides between +4183 and +4206 (5′-CAAGAGTTCGTTGTGCCTAAGTCA-3′) of the *dnaK* gene.

2.5. Detecting dnaK transcription by reverse transcriptase-mediated (RT)-PCR

RT-PCR was used to confirm the transcription of *dnaK*. An RT-PCR kit, the SuperScript one-step RT-PCR system (Gibco BRL Life Technologies), was used to amplify cDNA synthesized from *dnaK*-specific mRNA in total RNA, obtained from the culture shifted from 37°C to 42°C for 10 min. For the RT-PCR experiment, the primers were selected from the sequences of *dnaK*, open reading frame

PII: S0014-5793(99)00237-9

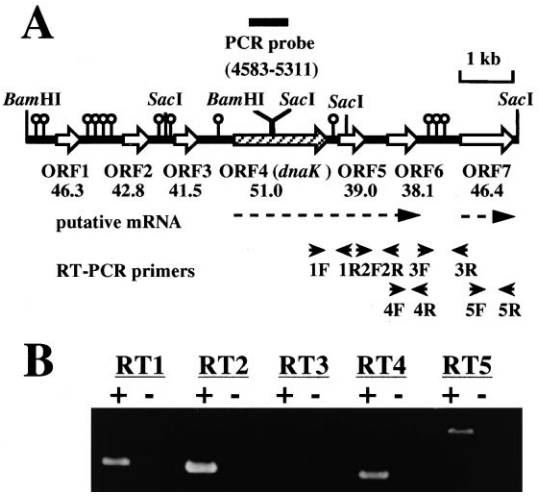

Fig. 1. Restriction map of the *P. gingivalis dnaK* locus and analysis of mRNA transcripts by RT-PCR. (A) The locations of the seven ORFs are indicated by arrows. The G+C content of each gene is given below the name of the respective gene. Open circles denote the inverted repeating units. The extents of the putative mRNA transcripts are shown below the DNA as broken lines. The short arrows indicate the primers for RT-PCR. (B) Gel electrophoresis of amplified DNA fragments. Lanes: RT1, amplification with primers 1F and 1R; RT2, amplification with primers 2F and 2R; RT3, amplification with primers 3F and 3R; RT4, amplification with primers 4F and 4R; RT5, amplification with primers 5F and 5R. Lanes marked with + are a standard amplification with RT-PCR. Lanes marked with − are negative control reactions that were heated at 94°C for 2 min prior to the addition of primers.

(ORF)5, ORF6 and ORF7. The pairs of oligonucleotide primers were: 1F, 5′-TTTACAAGAATGTCGGAG-3′ and 1R, 5′-AGATTGAAGTTGCGTTCT-3′; 2F, 5′-TGGCAATTATGTCTATGC-3′ and 2R, 5′-GTACCACATTTATTCCCT-3′; 3F, 5′-CTTACCACAACAATTGGT-3′ and 3R, 5′-AATGTTACATGCTCATCG-3′; 4F, 5′-ATGTACAAGCGACACAAA-3′ and 4R, 5′-TTAGAGTTTAGAAATCCA-3′; and 5F, 5′-ATGGCATACCAATCCAAG-3′ and 5R, 5′-TTATCCTACGGATGAGGA-3′. The experimental procedure was carried out according to the supplier's protocol. To check for the presence of contaminating DNA in the RNA preparation, the control reaction was initially heated at 94°C for 2 min prior to the addition of primers.

2.6. Constructing the fusion plasmid and expression and purification of the DnaK-intein-CBD fusion

A 1.7 kb DNA fragment that encoded the DnaK protein was amplified by PCR with a pair of primers (5′-GGGCATATGGGAAAAATCATTGGA-3′ and 5′-TTTCCCGGGTTTCACTTCCTCGAAGTC-3′) and chromosomal DNA from *P. gingivalis* ATCC 33277, followed by digestion with *Nde*I and *Sma*I. The resultant fragment was inserted into the *Nde*I-*Sma*I site of pTYB2 (New England Biolabs). The resulting plasmid, designated pKYB, was transformed into *E. coli* ER2566. Expression of the DnaK-YB fusion was induced by the addition of IPTG to a final concentration of 0.3 mM. Recombinant *P. gingivalis* DnaK was purified using the IMPACT T7 system (New England Biolabs).

2.7. ATPase assay

ATPase was assayed in a reaction mixture (100 μl) containing 25 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 150 mM KCl, 1 mM ATP and 0.6 μM DnaK homologue from *P. gingivalis*, 0.6 μM DnaJ from *E. coli* (StressGen) and various amounts of GrpE from *E. coli* (StressGen) [12]. After a 1 h incubation at 37°C, the reaction was terminated by adding 25 μl 20% perchloric acid. The reaction mixtures were centrifuged at 15 000 × *g* for 5 min at 4°C and the amount of inorganic phosphate released in the supernatant was measured by the method of Fiske and Subbarow [13] with modifications [14], using KH_2PO_4 as a standard.

3. Results

3.1. Isolation and nucleotide sequences of the dnaK locus from P. gingivalis

Four positive clones, screened by colony hybridization, were isolated and designated pMBLK1–4. Southern blot analysis of pMBLK3 revealed that 4.9 and 23 kb *Bam*HI fragments or 1.4 and 2.1 kb *Sac*I fragments hybridized with the *dnaK* probe (Fig. 1A). Sequencing of the 9878 bp fragment of pMBLK3 revealed seven contiguous ORFs (Fig. 1A). All of these ORFs had the same orientation. Sequence analysis of the region upstream of ORF4 revealed a putative promoter sequence resembling the *E. coli* σ^{70} consensus (Fig. 2A). We compared the putative amino acid (aa) sequences of the seven ORFs with known aa sequences stored in the international DNA databases (DDBJ/EMBL/GenBank). The deduced aa sequence of ORF4 showed a high homology to DnaK/HSP70 proteins of various other organisms. However, the ORFs upstream and downstream from ORF4 did not demonstrate any homology to *grpE*, *dnaJ* or the other heat shock genes. ORF4, which encoded a DnaK homologue, consisted of 641 aa, showed 59.3% identity and 84.8% similarity to *E. coli* DnaK (scores were calculated with CLUSTAL V [15]). The molecular weight of the DnaK homologue was estimated to be 69 136, which is close to the value determined by SDS-PAGE. Inverted repeat (IR) structures forming hairpin loops, defined as $\Delta G < -8.00$, were observed in the intergenic region (Fig. 1A). The average G+C content of all the sequenced regions was 46.0%. The highest G+C content was observed

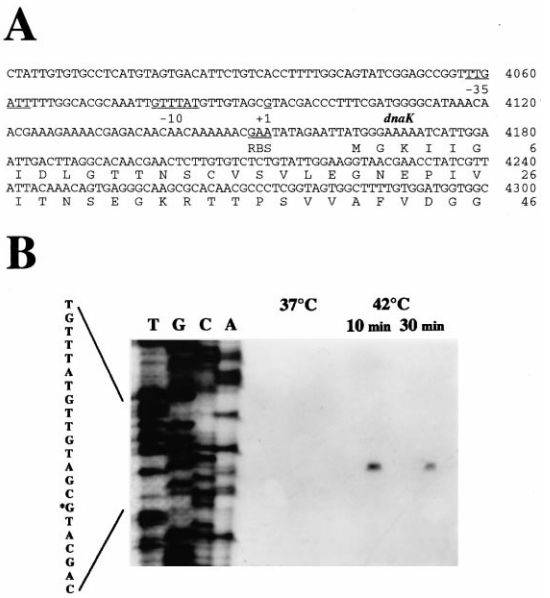

Fig. 2. (A) Nucleotide sequence of the promoter region of *dnaK* of *P. gingivalis*. The numbers to the right of the nucleotide sequence correspond to the position on a 9.9 kb *Bam*HI-*Sac*I fragment sequenced in this study. Putative Shine-Dalgarno boxes are marked by RBS. The putative −35 and −10 regions are underlined. The transcriptional start site of *dnaK* is underlined and labelled with +1. (B) Mapping the 5′ end of the *dnaK* operon by primer extension analysis. A primer complementary to the 5′ end of the *dnaK* gene was hybridized with total RNA from *P. gingivalis* isolated before and after incubation for 10 and 30 min at 42°C. A, C, G and T are products of the sequencing reaction obtained using the same primer. The sequence on the left is the antisense strand. An asterisk shows the transcriptional start position.

in *dnaK* (51.0%). The lowest G+C contents were observed in ORF5 (39.0%) and ORF6 (38.1%) (Fig. 1A).

3.2. Locating the transcription start site of the dnaK operon

The transcription start site of the *dnaK* operon was located by primer extension analysis with a primer complementary to the 5′ end of the *dnaK* gene. Total RNA from non-heat-shocked and heat-shocked cells was tested. *P. gingivalis* was grown anaerobically to the mid-exponential phase at 37°C. The culture was incubated at 37°C for 10 min and then it was shifted to 42°C for 10 or 30 min [16]. A control culture remained at 37°C. The transcriptional start site was in the same position under heat shock conditions and was located 70 bases upstream from the *dnaK* initiation codon, with a G at the 5′ end of the mRNA (Fig. 2A). It was located 9 bp downstream from the −10 region of the predicted promoter. A strong signal was obtained with RNA preparations from heat-shocked cells and the signal was even stronger after 10 min heat shock at 42°C than after 30 min (Fig. 2B).

3.3. Evidence for transcription of the dnaK locus in P. gingivalis

We used an RT-PCR experiment to identify the transcripts of *dnaK* and the other ORFs of *P. gingivalis*. Three pairs of oligonucleotide primers spanning the borders of *dnaK*/ORF5 (1F and 1R), ORF5/ORF6 (2F and 2R) and ORF6/ORF7 (3F and 3R) were designed (Fig. 1A). In addition, two pairs of primers were designed to amplify ORF6 (4F and 4R) and ORF7 (5F and 5R). Fig. 1B shows the amplified PCR products with two primer pairs, spanning *dnaK*/ORF5 and ORF5/ORF6. A DNA fragment within ORF6 and ORF7 was amplified. These amplified products had the expected sizes (685 bp with 1F and 1R, 653 bp with 2F and 2R, 617 bp with 4F and 4R and 1086 bp with 5F and 5R). PCR products corresponding to the regions spanning the borders of ORF6/ORF7 were not detected. No product was observed in PCRs from total RNA preparations that were not first reverse transcribed (Fig. 1B), indicating that the RT-PCR products were not derived from contaminated chromosomal DNA.

3.4. Purification of the P. gingivalis DnaK

We constructed a plasmid, pKYB, encoding *P. gingivalis* DnaK fused to the intein-CBD encoding sequence. Cell lysates prepared from *E. coli* ER2566 cells harboring the pKYB plasmid contained a fusion protein, DnaK-YB, which migrated at approximately 124 kDa on SDS-PAGE. The fusion protein was observed in the crude extract from cells induced with IPTG but not in that from not induced cells (data not shown). SDS-PAGE of recombinant *P. gingivalis* DnaK, purified using the IMPACT T7 system (New England Biolabs), showed a single 70 kDa protein band (data not shown). Approximately 10 mg of purified protein was obtained from 1 l of *E. coli* culture.

3.5. ATPase activity of the P. gingivalis DnaK

The ATPase activity of the purified recombinant DnaK of *P. gingivalis* was measured in the absence or presence of co-chaperones. The addition of *E. coli* DnaJ to the ATPase assay of *P. gingivalis* DnaK enhanced the activity roughly by 2-fold. The addition of both *E. coli* DnaJ and GrpE had a synergistic (about 5-fold) stimulatory effect on the ATPase activity of the *P. gingivalis* DnaK, although the addition of *E. coli* GrpE had no effect on the ATPase activity of the protein (Table 1). Various amounts of *E. coli* GrpE were added to an ATPase assay reaction mixture containing 0.6 μM *P. gingivalis* DnaK and 0.6 μM *E. coli* DnaJ. The ATPase activity was stimulated up to 40-fold when 3.6 μM or more GrpE was added to the reaction mixture (data not shown).

4. Discussion

The presence of DnaK in *P. gingivalis* has been proofed by Southern hybridization and by Western immunoblotting using rabbit anti-*E. coli* DnaK polyclonal antibodies [16]. However, the monoclonal anti-DnaK did not recognise DnaK protein in *P. gingivalis* [17]. This report describes the first characterization of the *dnaK* operon in a *Porphyromonas* species. The *dnaK* operon of *P. gingivalis* contains *dnaK* and two small heat shock genes with a novel arrangement, *dnaK-orf5-orf6*. Three types of *dnaK* operon have been reported to date. Many low G+C content Gram-positive bacteria possess *orfA-grpE-dnaK-dnaJ*. *dnaK-grpE-dnaJ-orfX* is only found in high G+C content Gram-positive bacteria while *dnaK-dnaJ*, which contains two distal genes of the basic organization, is found in α- and γ-purple proteobacteria [18]. A *dnaK* operon lacking both *grpE* and *dnaJ* has rarely been reported [19,20]. RT-PCR analysis of the *dnaK* gene of *P. gingivalis* revealed that ORF5 and ORF6 are indeed transcribed together with *dnaK* as one unit. Moreover, primer extension analysis demonstrated that the transcription of the operon is induced by heat shock. These findings indicate that the *dnaK* locus of *P. gingivalis* contains one *dnaK* gene and two small heat shock genes with the arrangement *dnaK-orf5-orf6*. The small heat shock proteins (sHSPs) belong to a ubiquitous family of low molecular mass (15–30 kDa), stress-induced proteins in prokaryotes and eukaryotes. Whereas various sHSPs share weak sequence homologies, many sHSPs appear to be functionally and structurally related [21]. The *dnaK* operon which contains the genes encoding two sHSPs have not been reported. ORF5 consisted of 185 aa and is predicted to encode a protein of approximately 21 665 Da with a pI of 9.65. The ORF6 product consisting of 206 aa is predicted to be a protein of approximately 23 535 Da with a pI of 9.26.

The aa sequence of *P. gingivalis* DnaK was compared with that of various microorganisms. A phylogenetic tree was constructed from a set of 30 HSP70 sequences (Fig. 3.). Interestingly, the aa sequence of *P. gingivalis* DnaK was close to the

Table 1
Stimulation of the ATPase activity of *P. gingivalis* DnaK by DnaJ and GrpE from *E. coli*

Protein	ATPase activity[a]
P. gingivalis DnaK	2.24 ± 0.35
P. gingivalis DnaK+*E. coli* DnaJ	4.79 ± 0.33
P. gingivalis DnaK+*E. coli* GrpE	2.20 ± 0.24
P. gingivalis DnaK+*E. coli* DnaJ+*E. coli* GrpE	11.60 ± 0.27

[a]A unit of activity is defined as nmol phosphate released per min per mg of DnaK. Data are expressed as means ± S.E.M. ($n = 5$).

sequences of HSP70s from Gram-positive bacteria and was clearly separated from Gram-negative bacteria in the phylogenetic tree.

The G+C content of the *P. gingivalis dnaK* operon also implicates its characteristic features. In general, determination of the G+C ratio is useful in predicting a degree of genetic relatedness among bacterial species [22]. *P. gingivalis dnaK* had a higher G+C content (51.0%) than that (46–48%) of the total genomic DNA of *P. gingivalis* [23] (Fig. 1A). On the other hand, the G+C contents of *orf5* (39.0%) and *orf6* (38.1%) were lower than that of the total genomic DNA. The divergence in the G+C ratio between species is attributed to variation in the mutation rates of the (A/T) to (G/C) and (G/C) to (A/T) base pairs [22]. Therefore, the discrepancy in the G+C content within the *dnaK* locus suggests that the genes in this region have different origins or histories.

An IR structure found between *dnaK* and *orf5* also supports different origins for these genes. This IR structure contains a part of the canonical arrangement of TTAGCACTC-N_9-GAGTGCTAA found in the regulatory region of heat shock genes from many eubacteria and its underlined sequences are conserved among Gram-positive bacteria [18,24–30]. So far, this IR has only been found in the upstream region of the *groE* and *dnaK* operons or genes. In *P. gingivalis*, the IR is located in the downstream region of the *dnaK* gene or the upstream region of *orf5*. The *orf5-orf6* genes may have an additional promoter and may be regulated in the same way as heat shock genes accompanied by the conserved IR. This result also suggests that these genes in the *P. gingivalis dnaK* region have a genetically unique character.

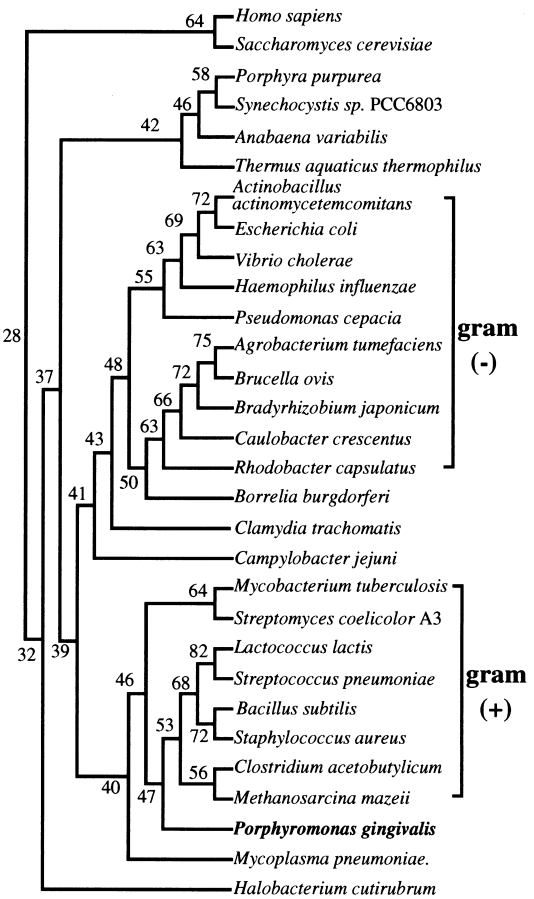

Fig. 3. Evolutionary tree, based on 30 HSP70 sequences, which included species representing the α-, β- and γ-subdivisions of purple bacteria, cyanobacteria, chlamydiae, spirochetes, both high and low G+C Gram-positive bacteria and mycoplasma, fungi and homo sapiens. The tree shown is a Neighbor-Joining distance tree constructed using the programme DNASIS Version 3.5. The phylogenetic analysis was carried out on the aa sequences of HSP70. The lengths of the branches do not indicate the phylogenetic distance.

To confirm that this *dnaK* homologue is not specific to *P. gingivalis* strain ATCC 33277, the *dnaK* homologue was amplified from several *P. gingivalis* strains by PCR. Chromosomal DNA from *P. gingivalis* W50, W83, 381, ATCC 33277 and ATCC 53977 was analyzed by PCR using a set of primers synthesized on the basis of the *dnaK* sequence from *P. gingivalis* ATCC 33277 (5′-ATGGGAAAAATCATTGGA-3′ and 5′-TTTCACTTCCTCGAAGTC-3′). A DNA fragment of similar size was amplified in all strains (data not shown).

In the folding of nascent polypeptides and denatured proteins, DnaK is known to bind to extended hydrophobic regions and to release them to prevent protein misfolding [4]. Each cycle of binding and release depends on the ATPase activity of DnaK. DnaJ stimulates the ATP hydrolytic activity of DnaK, allowing a complete cycle of peptide binding and release, whereas GrpE acts as a nucleotide exchanger to promote continued cycles of activity [31]. To rule out the possibility that this novel DnaK homologue lacks the enzymatic function of DnaK, the ATPase activity of *P. gingivalis* DnaK was examined in the presence or absence of DnaJ and GrpE from *E. coli*. Like the *E. coli* DnaK chaperone system [8,31], the ATPase activity of *P. gingivalis* DnaK is stimulated up to 40-fold in the presence of DnaJ and GrpE proteins from *E. coli* (data not shown), whereas *E. coli* DnaJ alone stimulates the ATPase activity of *P. gingivalis* DnaK only up to 2-fold (Table 1). The extent of stimulation increased with the amount of added *E. coli* GrpE and was saturated when 6 mol *E. coli* GrpE per mol of *P. gingivalis* DnaK and *E. coli* DnaJ was added (data not shown). In *E. coli* and *Thermus thermophilus*, titration of GrpE in the DnaK ATPase assay showed that components in the ratio of 1 mol of DnaK and DnaJ to about 2 mol of GrpE substantially stimulated the activity [31,32]. The different effects of GrpE in the DnaK ATPase assay may result from differences in the origin of DnaK and the other co-factors, DnaJ and GrpE. ATP hydrolysis is required for the efficient formation of a DnaK-DnaJ complex [33,34]. In our preliminary experiments, we confirmed that the ATPase activity of *P. gingivalis* DnaK was saturated when about 1 mol of *E. coli* DnaJ per mol of *P. gingivalis* DnaK was added (data not shown). These results indicate that the enzymatic activity of *P. gingivalis* DnaK is able to substitute for the *E. coli* DnaK activity, suggesting that *P. gingivalis* DnaK functions as a chaperone in the DnaK chaperone system. On the other hand, the previous report suggested that a large amount of DnaK is reactive heat-inactivated protein [35]. For the reason mentioned above, we cannot deny the possibility that DnaK acts alone as a chaperone.

As described above, it seems reasonable to suppose that DnaJ- and GrpE-like proteins exist in *P. gingivalis* on the other locus. Recently, a *P. gingivalis* W83 genome sequence database was released on the BLAST: Unfinished Microbial Genomes web site (http://www.ncbi.nlm.nih.gov/BLAST/unfinishedgenome.html). In this database, the gene *dnaK* homo-

logue and the two *dnaJ* and *grpE* homologues were found in different contigs. These facts support our results that the *dnaK* operon of *P. gingivalis* possesses a unique organization.

In conclusion, *P. gingivalis* DnaK is unique among the known bacterial HSP70s in several respects: (i) its operon has a unique gene arrangement, (ii) there are two genes which encode small heat shock proteins in its operon and (iii) it is distant from Gram-negative bacteria rather than Gram-positive bacteria in the phylogenetic tree. In spite of this uniqueness, *P. gingivalis* DnaK cooperated with *E. coli* DnaJ and GrpE. Further studies on the *P. gingivalis* DnaK chaperone system will clarify the mechanisms that respond to oral conditions such as temperature and pH.

Acknowledgements: This work was supported in part by Grants-in-aid of Scientific Research number 10307054 and 08457572 from the Ministry of Education, Science, Sports and Culture, Tokyo, Japan and by a research grant from the Fund for Comprehensive Research on Aging and Health.

References

[1] Lindquist, S. and Craig, E.A. (1988) Annu. Rev. Genet. 22, 631–677.
[2] Gupta, R.S. and Golding, G.B. (1993) J. Mol. Evol. 37, 573–582.
[3] Georgopoulos, C. (1992) Trends Biochem. Sci. 17, 295–299.
[4] Langer, T., Lu, C., Echols, H., Flanagan, J., Hayer, M.K. and Hartl, F.U. (1992) Nature 356, 683–689.
[5] Hendrick, J.P., Langer, T., Davis, T.A., Hartl, F.U. and Wiedmann, M. (1993) Proc. Natl. Acad. Sci. USA 90, 10216–10220.
[6] Thomas, J.G., Ayling, A. and Baneyx, F. (1997) Appl. Biochem. Biotechnol. 66, 197–238.
[7] Tsai, J. and Douglas, M.G. (1996) J. Biol. Chem. 271, 9347–9354.
[8] Szabo, A., Langer, T., Schroder, H., Flanagan, J., Bukau, B. and Hartl, F.U. (1994) Proc. Natl. Acad. Sci. USA 91, 10345–10349.
[9] Slots, J. and Genco, R.J. (1984) J. Dent. Res. 63, 412–421.
[10] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[11] Nakano, Y., Yoshida, Y., Yamashita, Y. and Koga, T. (1995) Gene 162, 157–158.
[12] Motohashi, K., Yohda, M., Odaka, M. and Yoshida, M. (1997) FEBS Lett. 412, 633–636.
[13] Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400.
[14] Lill, R., Dowhan, W. and Wickner, W. (1990) Cell 60, 271–280.
[15] Higgins, D.G. and Sharp, P.M. (1988) Gene 73, 237–244.
[16] Lu, B. and McBride, B.C. (1994) Oral Microbiol. Immunol. 9, 166–173.
[17] Vayssier, C., Mayrand, D. and Grenier, D. (1994) FEMS Microbiol. Lett. 121, 303–307.
[18] Segal, G. and Ron, E.Z. (1996) FEMS Microbiol. Lett. 138, 1–10.
[19] Chitnis, P.R. and Nelson, N. (1991) J. Biol. Chem. 266, 58–65.
[20] Deckert, G., Warren, P.V., Gaasterland, T., Young, W.G., Lenox, A.L., Graham, D.E., Overbeek, R., Snead, M.A., Keller, M., Aujay, M., Huber, R., Feldman, R.A., Short, J.M., Olsen, G.J. and Swanson, R.V. (1998) Nature 392, 353–358.
[21] Veinger, L., Diamant, S., Buchner, J. and Goloubinoff, P. (1998) J. Biol. Chem. 273, 11032–11037.
[22] Sueoka, N. (1988) Proc. Natl. Acad. Sci. USA 85, 2653–2657.
[23] Shah, H.N. (1993) in: Biology of the Species *Porphyromonas gingivalis* (Shah, H.N., Mayrand, D. and Genco, R., Eds.), pp. 59–82, CRC Press, Boca Raton, FL.
[24] Kim, S.G. and Batt, C.A. (1993) Gene 127, 121–126.
[25] van Asseldonk, M., Simons, A., Visser, H., de Vos, W.M. and Simons, G. (1993) J. Bacteriol. 175, 1637–1644.
[26] Schmidt, A., Schiesswohl, M., Volker, U., Hecker, M. and Schumann, W. (1992) J. Bacteriol. 174, 3993–3999.
[27] Wetzstein, M., Wetzstein, M., Volker, U., Dedio, J., Lobau, S., Zuber, U., chiesswohl, M., Herget, C., Hecker, M. and Schumann, W. (1992) J. Bacteriol. 174, 3300–3310.
[28] Webb, R., Reddy, K.J. and Sherman, L.A. (1990) J. Bacteriol. 172, 5079–5088.
[29] Narberhaus, F. and Bahl, H. (1992) J. Bacteriol. 174, 3282–3289.
[30] Narberhaus, F., Giebeler, K. and Bahl, H. (1992) J. Bacteriol. 174, 3290–3299.
[31] Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C. and Zylicz, M. (1991) Proc. Natl. Acad. Sci. USA 88, 2874–2878.
[32] Motohashi, K., Taguchi, H., Ishii, N. and Yoshida, M. (1994) J. Biol. Chem. 269, 27074–27079.
[33] Wawrzynów, A. and Zylicz, M. (1995) J. Biol. Chem. 270, 19300–19306.
[34] Wawrzynów, A., Banecki, B., Wall, D., Liberek, K., Georgopoulos, C. and Zylicz, M. (1995) J. Biol. Chem. 270, 19307–19311.
[35] Ziemienowicz, A., Skowyra, D., Zeilstra-Ryalls, J., Fayet, O., Georgopoulos, C. and Zylicz, M. (1993) J. Biol. Chem. 268, 25425–25431.